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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
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ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 05/19/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/673,448	CLARK ET AL.	
	Examiner	Art Unit	
	Jeanine A Goldberg	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 February 2003* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-50* is/are pending in the application.

4a) Of the above claim(s) *49 and 50* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-48* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

1. This action is in response to the papers filed February 6, 2003. Currently, claims 1-50 are pending. Claims 49-50 have been withdrawn as drawn to non-elected subject matter.

2. All arguments have been thoroughly reviewed but are deemed non-persuasive for the reasons which follow. This action is made FINAL.

3. Any objections and rejections not reiterated below are hereby withdrawn in view of the amendments to the claims or applicant's remarks.

New Grounds of Rejection Necessitated by Amendment

New Matter

4. Claims 1-48 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

In the amended claims, reference to "represented by nucleotides 1-98 of SEQ ID NO: 52-54" are included. The amendment does not appear to point to support for the new claim language. The specification does not describe or discuses "represented by nucleotides 1-98 of SEQ ID NO: 52-54". The specification does not appear to contemplate nucleotides 1-98 of SEQ ID NO: 52-54. Each of these sequences are between 400-550 nucleotides in length. The concept of "represented by nucleotides 1-98 of SEQ ID NO: 52-54" does not appear to be part of the originally filed invention.

The specification does not appear to specifically delineate nucleotides 1-98 of these sequences. Therefore, "represented by nucleotides 1-98 of SEQ ID NO: 52-54" constitutes new matter. Applicant is required to cancel the new matter in the reply to this Office Action.

Maintained Rejections

Priority

5. This application claims priority to PCT/AU99/00306, filed April 23, 1999 and Australian PP 3129, filed April 23, 1998.

It is noted that the priority document does not appear to contain support for differential methylation in liver cancer tissue DNA extracts. Figure 9 of the instant application appears to be first present in the PCT/AU99/00306. Therefore, Claims directed to liver cancer are not supported by the Australian PP 3129 document and receive the benefit of April 23, 1999.

The examiner has reviewed the priority document with respect to the presence of liver cancer. While there is no intervening art on record and therefore the observation does not appear to affect any of the rejections of record, the teachings in the priority document do not provide an enabling disclosure that liver cancer contains differential methylation.

Information Disclosure Statement

6. The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other

information submitted for consideration by the Office, and MPEP § 609 A(1) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on form PTO-892, they have not been considered.

Claim Rejections - 35 USC § 112-Scope of Enablement

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

7. Claims 1-16, 22-23, 35-46 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods of detecting prostate or liver cancer by differential methylation analysis of the GST-Pi gene, does not reasonably provide enablement for method of detecting any cancer based upon differential methylation analysis of the GST-Pi gene. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988). *Wands* states at page 1404,

> "Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the

relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The claims are broadly drawn to method of detecting any cancer based upon differential methylation analysis of the GST-Pi gene.

The specification and the art support the differential methylation analysis of the GST-Pi gene as indicative of prostate cancer and liver cancer (Figure 9)(see Lee, Nelson, for example).

Lee et al. (herein referred to as Lee) teaches CG island methylation changes near the GSTP1 gene in prostatic carcinoma cells detected using the polymerase chain reaction: a new prostate cancer biomarker. Lee teaches isolating DNA from normal and neoplastic cells and tissue from prostate carcinoma specimens (page 443, col. 2)(limitations of Claim 1 (i), 14-17, 44-47). Lee teaches that the DNA specimens were subjected first to exhaustive digestion with excess HpaII, which cuts the unmethylated sequence (page 443, col. 2). The samples were then amplified using PCR primers (page 443, col. 2)(limitations of Claim 3). As seen in Figure 1, GSTP hypermethylation of the promoter region, -408 to +197 was amplified in the describe method and then further amplified by primers directed to –220 to -57, but GSTP1 hypomethylation in the same region did not yield a PCR product, i.e., the amplification is selective that it only amplifies the target region if the site at which abnormal cytosine methylation occurs is methylated (Claim 1 (ii), 18-25, 35-39). The PCR amplification products are then electrophoresed on polyacrylamide gels and visualized by staining with ethidium bromide or Southern blot hybridization analysis (page 444, col. 1)(limitations of Claim 1

(iii)). Lee teaches that using the PCR assay strategy, more than 90% of human prostatic carcinoma DNA specimens analyzed exhibited deoxycytidine methylation changes extensively encompassing the GSTP1 promoter region (page 443, col. 2).

Nelson teaches the hypermethylation of GSTP1 expression in prostate cancer.

Bakker et al (J. of Biological Chemistry, Vol. 277, No. 25, pages 22573-22580, June 2002) teaches GST-Pi becomes hypermethylated during hepatocellular carcinoma (HCC). Bakker teaches many clones are hypermethylated at the examined regions and show hypermethylation.

Esteller et al. (Cancer Reserach, Vol. 58, pages 4515-4518, October 1998) teaches that GSTP1 is hypermethylated most frequently in breast and renal carcinomas, showing aberrant methylation in 30 and 20% of the cases, respectively (abstract). Esteller also teaches examining aberrant methylation using MSP in tumors including breast, endometrial, ovarian, renal, bladder, colon, pancreatic, lung, head and neck carcinoma, melanomas, leukemias, lymphomas, gliomas and meningiomas (page 4515, col. 2). As seen in Table 1, GSTP1 promoter hypermethylation was not found in any of the endometrial, ovarian, meningioma, melanoma, leukemias, head and neck carcinoma, bladder or pancreatic carcinomas (pager 4516, col. 2).

Virmani et al (Clinical Cancer Research, Vol. 7, No. 3, pages 584-589, March 2001) teaches aberrant methylation during cervical carcinogenesis. Virmani teaches that GSTP1 methylation was an early event in carcinogenesis (abstract). As seen in Table 1, frequency of aberrant gene methylation during cervical carcinogenesis is

illustrated. Methylation was found in 11% of nondysplasia/low grade CIN; 18% of high grade CIN; 21% of invasive cancer; and 30% of cervical cancer cell lines (page 586).

Melki et al. (Cancer Research, Vol. 59, pages 3730-3740, August 1999) teaches hypermethylation status in several genes in individuals with acute myeloid leukemia (AML). As seen in Figure 7B, GST-Pi was analyzed and an equal proportion of patients who were normal and who had AML methylation of GST-Pi. As see in Figure 7A, none of the patients showed methylation of GST-Pi. Therefore, GST-Pi does not appear to be differentially methylated in AML patients as compared with normal controls.

Neither the specification nor the art teach the skilled artisan how to use the invention as broadly as claimed. The claims broadly encompass any disease or condition, including cancers or specifically breast cancer, cervical cancer and liver cancer (Claims 1, 16, for example). First, conditions or diseases encompasses diabetes, AIDs, balding, obesity, hair colors and cardiovascular diseases, for example. Each of these conditions are not associated with differential methylation patterns of the GST-Pi gene. The skilled artisan, would be required undue experimentation to determine which of the innumerable diseases and conditions are associated with the differential methylation patterns of the GST-Pi gene. While one could conduct additional experimentation to determine whether, e.g. differential methylation patterns of the GST-Pi gene, might be associated with, e.g., certain types of cardiovascular disease, hair colors, or other health conditions, the outcome of such research cannot be predicted, and such further research and experimentation are both unpredictable and undue. With respect to cancers generally, cancers are also a diverse classification of

diseases which includes leukemias, breast, prostate, ovarian, liver, cervical, lung, AML among others. There is no evidence that each of these cancers function in the same manner, in fact, the art suggests that different genes, and mechanisms affect the different cancers. Based upon the analysis performed by Esteller, GSTPi is not differentially expressed in all cancers. It is unclear which cancers may be diagnosed/prognosed based upon differential GSTPi methylation without further experimentation. Therefore, absent specific teachings of differential methylation patterns of the GST-Pi gene, it is unpredictable that GST-Pi is differentially expressed in additional cancers. Thus, it is unpredictable as to whether one could successfully use the claimed invention, and given the fact that neither the specification nor the prior art provide evidence of a correlation or association between differential methylation patterns of the GST-Pi gene with diseases or conditions. It is further unpredictable as to whether any quantity of experimentation would allow one to practice the claimed invention. Accordingly, it would require undue experimentation for a skilled artisan to use the claimed invention.

Response to Arguments

The response traverses the rejection. The response asserts that the claims have been amended to cancers characterized by abnormal methylation of cytosine (page 13 of response filed February 6, 2003). This argument has been reviewed but is not convincing because the enablement rejection and the art states that not all cancers are associated with abnormal methyaltion. As seen in Table 1 of Esteller, GSTP1 promoter hypermethylation was not found in any of the endometrial, ovarian, meningioma,

melanoma, leukemias, head and neck carcinoma, bladder or pancreatic carcinomas (pager 4516, col. 2). As see in Figure 7A of Melki, none of the patients showed methylation of GST-Pi. Therefore, GST-Pi does not appear to be differentially methylated in AML patients as compared with normal controls. With respect to cancers generally, cancers are also a diverse classification of diseases which includes leukemias, breast, prostate, ovarian, liver, cervical, lung, AML among others. There is no evidence that each of these cancers function in the same manner, in fact, the art suggests that different genes, and mechanisms affect the different cancers. Based upon the analysis performed by Esteller, GSTPi is not differentially expressed in all cancers. It is unclear which cancers may be diagnosed/prognosed based upon differential GSTPi methylation without further experimentation. Therefore, absent specific teachings of differential methylation patterns of the GST-Pi gene, it is unpredictable that GST-Pi is differentially expressed in additional cancers. Thus, it is unpredictable as to whether one could successfully use the claimed invention, and given the fact that neither the specification nor the prior art provide evidence of a correlation or association between differential methylation patterns of the GST-Pi gene with diseases or conditions. It is further unpredictable as to whether any quantity of experimentation would allow one to practice the claimed invention. Accordingly, it would require undue experimentation for a skilled artisan to use the claimed invention.

Thus for the reasons above and those already of record, the rejection is maintained.

Claim Rejections - 35 USC § 112- Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

8. Claims 1-48 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

B) Claims 1-48 are indefinite because it is unclear what "within the region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) GpG sites –43 to +55" encompasses. It is unclear whether the amplified by the method is defined by –43 to +55 such that these are the boundaries of the amplified region, or whether the region must merely include these CpG sites. Moreover, it is unclear what is meant by –43 to +55 because there is no frame of reference. There is no relative position to which these sites may be compared. This rejection could be overcome by reciting nucleotide positions within a SEQ ID NO: of the application. However, as written, the metes and bounds of the claimed invention are unclear.

Response to Arguments

The response traverses the rejection. The response asserts that the claim has been amended to recite "represented by nucleotides 1-98 of SEQ ID NO: 52-54." This argument has been reviewed but is not convincing because it is unclear how the sites may be represented by three nucleotide sequences. Thus for the reasons above and those already of record, the rejection is maintained.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

9. Claims 1, 3, 14-25, 35-39, 44-47 are rejected under 35 U.S.C. 102(b) as being anticipated by Lee et al (Cancer Epidemiology, Biomarkers, Prevention. Vol. 6, pages 443/450, June 1997).

Lee et al. (herein referred to as Lee) teaches CG island methylation changes near the GSTP1 gene in prostatic carcinoma cells detected using the polymerase chain reaction: a new prostate cancer biomarker. Lee teaches isolating DNA from normal and neoplastic cells and tissue from prostate carcinoma specimens (page 443, col. 2)(limitations of Claim 1 (i), 14-17, 44-47). Lee teaches that the DNA specimens were subjected first to exhaustive digestion with excess HpaII, which cuts the unmethylated sequence (page 443, col. 2). The samples were then amplified using PCR primers (page 443, col. 2)(limitations of Claim 3). As seen in Figure 1, GSTP hypermethylation of the promoter region, -408 to +197 was amplified in the describe method and then further amplified by primers directed to –220 to -57, but GSTP1 hypomethylation in the same region did not yield a PCR product, i.e., the amplification is selective that it only amplifies the target region if the site at which abnormal cytosine methylation occurs is methylated (Claim 1 (ii), 18-25, 35-39). The PCR amplification products are then

electrophoresed on polyacrylamide gels and visualized by staining with ethidium bromide or Southern blot hybridization analysis (page 444, col. 1)(limitations of Claim 1 (iii)). Lee teaches that using the PCR assay strategy, more than 90% of human prostatic carcinoma DNA specimens analyzed exhibited deoxycytidine methylation changes extensively encompassing the GSTP1 promoter region (page 443, col. 2).

Response to Arguments

The response traverses the rejection. The response asserts that the amplification step is "selective in that it only amplifies the target region if the said sites or sites at which abnormal cytosine methylation occurs is/are methylated." The response further states that Lee relies on a preceding exhaustive digestion step which is selective. Therefore, the argument has been reviewed but is not convincing because the presence of a preceding exhaustive digestion step which is selective prior to amplification inherently allows the amplification to be selective in that it only amplifies the target region if the said sites or sites at which abnormal cytosine methylation occurs is/are methylated. As seen in Figure 1, following the cleavage, there is no amplification product formed in the hypermethylation nucleic acid. Therefore, the amplification using Primer set A and Primer set B is inherently selective to methylation since PCR does not occur after the cleavage step. The claims as written are broadly drawn to comprising methods which allow for additional steps, therefore the additional step of digestion prior to amplification is encompassed by the instant claims. Thus for the reasons above and those already of record, the rejection is maintained.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

10. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

11. Claims 2, 4-13, 40-41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lee et al (Cancer Epidemiology, Biomarkers, Prevention. Vol. 6, pages 443/450, June 1997) in view of Herman et al. (US Pat. 5,786,146, July 1998).

Lee et al. (herein referred to as Lee) teaches CG island methylation changes near the GSTP1 gene in prostatic carcinoma cells detected using the polymerase chain reaction: a new prostate cancer biomarker. Lee teaches isolating DNA from normal and neoplastic cells and tissue from prostate carcinoma specimens (page 443, col. 2)(limitations of Claim 1 (i), 14-17, 44-47). Lee teaches that the DNA specimens were

subjected first to exhaustive digestion with excess HpaII, which cuts the unmethylated sequence (page 443, col. 2). The samples were then amplified using PCR primers (page 443, col. 2)(limitations of Claim 3). As seen in Figure 1, GSTP hypermethylation of the promoter region, -408 to +197 was amplified in the describe method and then further amplified by primers directed to –220 to -57, but GSTP1 hypomethylation in the same region did not yield a PCR product, i.e., the amplification is selective that it only amplifies the target region if the site at which abnormal cytosine methylation occurs is methylated (Claim 1 (ii), 18-25, 35-39). The PCR amplification products are then electrophoresed on polyacrylamide gels and visualized by staining with ethidium bromide or Southern blot hybridization analysis (page 444, col. 1)(limitations of Claim 1 (iii)). Lee teaches that using the PCR assay strategy, more than 90% of human prostatic carcinoma DNA specimens analyzed exhibited deoxycytidine methylation changes extensively encompassing the GSTP1 promoter region (page 443, col. 2).

Lee suggests that bisulfite detection may prove useful as molecular staging and diagnosis strategies (page 449, col. 1). Moreover, Lee teaches that methylated CG dinucleotides can be distinguished from CG dinucleotides at specific genomic DNA loci by several means potentially amenable to use with DNA amplification strategies including treating amplified DNA with bisulfite to promote selective deamination of C nucleotides to U nucleotides that permits discrimination of C nucleotides from methylated C nucleotides as differences in the nucleotide sequence of the amplification product accompanying bisulfite treatment (page 446, col. 2). Lee does not specifically

teach the benefit of using bisulfite treatment for distinguishing methylated DNA from unmethylated DNA.

However Herman et al. (herein referred to as Herman) teaches methylation specific PCR (MSP) for rapid identification of DNA methylation patters in a CpG containing nucleic acid (abstract). Herman reviews numerous ways that methylation had been previously detected, including methylation-sensitive enzymes, Southern hybridization with methylation sensitive restriction enzymes and methylation sensitive enzymes and the PCR (col. 2-3). Herman teaches that each of these methods have drawbacks which makes utizilizing bisulfite treatment of DNA to convert all unmethylated cytosines to uracil followed by PCR advantageous. Herman teaches a method for rapid assessment of the methylation status of any group of CpG sites within a CpG island independent of the use of methylation-sensitive restriction enzymes (col. 3, lines 40-45). MSP requires only small amounts of DNA, is sensitive to 0.1% of methylated alleles of a given CpG island locus, and can be performed on DNA extracted from paraffin-embedded samples (col. 3, lines 50-55). MSP eliminates the false positive results inherent to previous PCR-based approaches which relied on differential restriction enzyme cleavage to distinguish methylated from unmethylated DNA (col. 3, lines 55-58). Herman teaches that MSP primers are specifically designed to recognize CpG sites to take advantage of the differences in methylation to amplify specific products to be identified by the invention assay (col. 4, lines 55-60). Herman compares MSP to the only technique that can provide more direct analysis, namely genomic sequencing. MSP is much simpler and requires less time than genomic sequencing,

avoids the use of expensive sequencing reagents and the use of radioactivity, and increased sensitivity (col. 5, liens 15-30). The MSP method comprises contacting a methylated CpG containing nucleic acid specimen with an agent that modifies unmethylated cytosine, amplifying the CpG containing nucleic acid in the specimen by means of CpG-specific oligonucleotide primers and detecting the methylated nucleic acid (col. 5, lines 40-47). Herman teaches that the preferred agent for modifying unmethyalted cytosine is sodium bisulfite (limitations of Claim 2, 8, 40-41). Herman teaches that cytosine reacts with the bisulfite ion to form a sulfonated cytosine reaction intermediate which is susceptible to deamination, giving rise to a sulfonated uracil (col. 5, lines 60-62). Uracil is recognized as a thymine by Taq polymerase and therefore upon PCR, the resultant product contains cytosine only at the position where 5-methylcytosine occurs in the starting template DNA (col. 5, lines 63-67)(limitations of Claim 4-5). Herman teaches that the method of amplifying is by PCR preferably (col. 8, lines 58-59)(limitations of Claim 9). Herman teaches that the primers specifically distinguish between untreated DNA, methylated and non-methylated DNA. MSP primers usually contain relatively few Cs or Gs in the sequence since the Cs will be absent in the sense primer and Gs absent in the antisense primer (col. 6, lines 5-10)(limitations of Claim 7, 10, 11, 13). The primers typically contain 12-20 or more nucleotides, although they may contain fewer nucleotides (col. 6, lines 34-37)(limitations of Claim 6, 12). Herman teaches that any specimen in purified or nonpurified form can be used. The specimen may be from any source including prostate, lung (col. 7, lines 30-35). The nucleic acid is in the region of the promoter of a structural gene typically

(col. 10, lines 17-19). Herman teaches that the detection of the methylated CpG containing nucleic acid in the specimen may be indicative of cellular proliferative disorder or neoplasia including prostate cancer.

Therefore, it would have been prima facie obvious to one of ordinary skill in the art to have modified the methylation detection method of Lee with the methylation detection method of Herman. The ordinary artisan would have realized based upon the explicit teachings of Herman, that the method of using methylation specific primers was advantageous over digestion with methylation-sensitive enzymes followed by PCR. Herman specifically states, "MSP eliminates the false positive results inherent to previous PCR-based approaches which relied on differential restriction enzyme cleavage to distinguish methylated from unmethylated DNA". Herman also additional advantages of using MSP detection methods as compared to other methylation detection methods. Since Herman has specifically compared the methylation detection method of Lee with the modified methylation detection method of Herman and found that the MSP methylation detection method has benefits, the ordinary artisan would have been motivated to have used the MPS methylation detection method. Therefore, the ordinary artsian would have been motivated to have modified the method of detecting methylation in the promoter region of Lee with the MSP methylation detection method of Herman for the explicit benefits taught by Herman.

Response to Arguments

The response traverses the rejection. The response asserts that Lee does not teach nor suggest the present invention. This argument has been reviewed but is not

convincing for the reasons presented above. Furthermore, combining the methods of Lee and Herman would be directed to a selective amplification method.

The response argues that Herman detection method is based upon primers that an distinguish between methylated and unmethylated CpG sites. The response asserts that this is not abnormal methylation. This argument has been thoroughly reviewed, but is not found persuasive because abnormal methylation causes normally unmethylated CpG sites to become hypermethylated in disease states or alternatively normally methylated CpG sites to become hypomethylated. Therefore, methylation and unmethylation are indicative of abnormal methylation patterns.

The response focuses on the ability of Lee to simultaneously detect all 12 recognition sequences for HpaII and MSP1 in a sample DNA. The response further poses the question of what the result would be for the modification of detecting methylation in the promoter region of Lee with the MSP method of Herman. The response points out, correctly, that the primers need to be in close proximity to the CpG sites. The ordinary artisan would have clearly recognized this aspect of the MSP method from the teachings of Herman. Moreover, the teachings of Lee include methylation CpG sites over a large region. The ordinary artisan would have recognized that several different amplifications may need to be performed to analyze the complete region. Herman teaches examining regional aspects of CpG island methylation (col. 14). Herman also teaches that MSP allows examination of all CpG sites, not just those within sequences recognized by methyaltion sentisitve enzymes; MSP elemintates the frequent results due to partial digeetions. Moreover, Herman specifically compared the

methylation detection method of Lee with the modified methylation detection method of Herman and found that the MSP methylation detection method has benefits, the ordinary artisan would have been motivated to have used the MPS methylation detection method. The suggested "primer" of several hundred nucleotides in length is not how Herman solves the problem of multiple CpG sites. The primers of Herman "preferably have a T in the 3' CG pair to distinguish it form the C retained in the methylated DNA." Therefore, it is clear from Herman that various primer pairs are used to distinguish between methylated or unmethylated, i.e. abnormal methylation. A single primer is not used to distinguish all recognition sites in a promoter region as suggested by the response.

The response asserts that the ordinary artisan would "not have seen the relevance of Herman's MSP method based on oligonucleotide primers to the detection of the extensive methylation marker taught by Lee et al." (page 19 of response). This argument has been thoroughly reviewed, but is not found persuasive because Herman teaches using multiple primer pairs for different methylation sites. Moreover, Lee strongly suggests that using bisulfite treated DNA can discriminate between C nucleotides and methylated C nucleotides as differences in amplification. Therefore, given the expected benefits taught by Herman for using MSP, the ordinary artisan would have been motivated and expected use the improved MSP method over the digestion methods.

Lee teaches that the CpG sites within the region of the GST-Pi gene and/or its regulatory flanking region can be sued as a marker for prostate cancer. Furthermore,

the claims are not limited to the region of nucleotides 1-98 of SE QID NO: 52-54, but are directed to the region o the gene or regulatory flanking sequences defined by nucleotides 1-98 of SEQ ID NO: 52-54. Therefore, the regions taught by Lee fall within the broad scope of both the gene and the regulatory flanking region.

Thus for the reasons above and those already of record, the rejection is maintained.

12. Claims 26-29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lee et al (Cancer Epidemiology, Biomarkers, Prevention. Vol. 6, pages 443/450, June 1997) in view of Herman et al. (US Pat. 5,786,146, July 1998) as applied to Claims 2, 4, 13, 40-41 above and further in view of Jhaveri (Gene, Vol. 210, pages 1-7, March 1998) and Morrow et al (Genbank Accession Number M24485, December 1994).

Neither Lee nor Herman teach the specific primers for the amplification of the CpG island of GST-Pi.

However, Jhaveri et al. (herein referred to as Jhaveri) teaches the regions of GST-Pi which are methylated. Jhaveri teaches that the CpG island spans the proximal promoter and the first and second exon and intron.

Morrow teaches the full GST-Pi sequence which includes the proximal promoter, the first and second exon and intron.

Therefore, it would have been prima facie obvious to one of ordinary skill in the art to have modified the primers taught by Lee to obtain additional primers for amplification of the CpG island. The ordinary artisan would have recognized based

upon the teachings of Jhaveri that one would desire amplifying the proximal promoter, first and second exon and intron. Given the full GST-Pi sequence, the ordinary artisan would have been able to have generated primers which flank these sequences or are directed to specific subsequences within the CpG island for amplification and analysis of the CpG island which allows diagnosis of prostate cancers. Therefore, the instantly claimed primer pairs are functional equivalents to the primer pairs taught by Lee in view of Herman. Herman teaches the general design of MSP primers. Namely, Herman teaches MSP primers usually contain relatively few Cs or Gs in the sequence since the Cs will be absent in the sense primer and Gs absent in the antisense primer (col. 6, lines 5-10). The primers typically contain 12-20 or more nucleotides, although they may contain fewer nucleotides (col. 6, lines 34-37). Therefore, taking the primers taught by Lee in view of Herman and obtaining alternative functional equivalents which may also amplify the CpG island regions of interest, taught by Jhaveri, would have been well within the guidance provided in the art for the ordinary artisan. The art provides a specific region to design primers to for the detection of methylation, provides how to generate primers which will differentiate methylated nucleic acids from unmethylated nucleic acids and provides a clear advantage of using MSP primers for the differential methylation detection. Therefore, the instant primers of SEQ ID NO: 1-16 are merely functional equivalents for those already taught in the art. Since the claimed oligonucleotides simply represent functional equivalents concerning which a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties,

the claimed primers and probes are *prima facie* obvious over the cited reference in the absence of secondary considerations.

Response to Arguments

The response traverses the rejection. The response asserts that Jhaveri and Morrow do not provide the deficiencies from Lee and Herman. This argument has been reviewed but is not convincing for the reasons discussed above.

Thus for the reasons above and those already of record, the rejection is maintained.

13. Claims 30-34, 48 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lee et al (Cancer Epidemiology, Biomarkers, Prevention. Vol. 6, pages 443/450, June 1997) in view of Tchou et al (Hepatology, Vol. 28, No. 4, pages 47, October 1998).

Lee et al. (herein referred to as Lee) teaches CG island methylation changes near the GSTP1 gene in prostatic carcinoma cells detected using the polymerase chain reaction: a new prostate cancer biomarker. Lee teaches isolating DNA from normal and neoplastic cells and tissue from prostate carcinoma specimens (page 443, col. 2)(limitations of Claim 1 (i), 14-17, 44-47). Lee teaches that the DNA specimens were subjected first to exhaustive digestion with excess HpaII, which cuts the unmethylated sequence (page 443, col. 2). The samples were then amplified using PCR primers (page 443, col. 2)(limitations of Claim 3). As seen in Figure 1, GSTP hypermethylation of the promoter region, -408 to +197 was amplified in the describe method and then further amplified by primers directed to –220 to -57, but GSTP1 hypomethylation in the same region did not yield a PCR product, i.e., the amplification is selective that it only

amplifies the target region if the site at which abnormal cytosine methylation occurs is methylated (Claim 1 (ii), 18-25, 35-39). The PCR amplification products are then electrophoresed on polyacrylamide gels and visualized by staining with ethidium bromide or Southern blot hybridization analysis (page 444, col. 1)(limitations of Claim 1 (iii)). Lee teaches that using the PCR assay strategy, more than 90% of human prostatic carcinoma DNA specimens analyzed exhibited deoxycytidine methylation changes extensively encompassing the GSTP1 promoter region (page 443, col. 2).

Lee does not specifically teach detecting hypermethylation in GST-Pi as an indicator of liver cancer (hepatocellular carcinoma).

However, Tchou et al. (herein referred to as Tchou) teaches the role of GST-Pi expression in hepatocarcinogenesis. Given the teachings that GST-Pi is hypermethylated in prostate cancer, Tchou hypothesized the same phenomenon may occur in HCC. Tchou teaches that CpG methylation is a common phenomenon in HCC. Using PCR-based methylation assay, none of the normal tissues have evidence of CpG methylation near the promoter, however, 18 of 20 tumors showed methylation in that region.

Therefore, it would have been prima facie obvious to one of ordinary skill in the art to have modified the teachings of Lee for detecting prostate cancer based upon differential methylation of GST-Pi with the teachings of Tchou that GST-Pi is also hypermethylated in hepatocellular carcinoma. Therefore, the ordinary artisan would have been motivated to have applied the method for screening for liver cancer using the same assay as taught in the art for prostate cancer. The ordinary artisan would have

been able to have taken samples from the two organs and performed analysis on each of the samples to obtain a more comprehensive analysis of the patients cancer status.

Response to Arguments

The response traverses the rejection. The response asserts that Tchou does not provide the deficiencies from Lee and Herman. This argument has been reviewed but is not convincing for the reasons discussed above.

Thus for the reasons above and those already of record, the rejection is maintained.

Conclusion

14. No claims allowable over the art.

15. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

16. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Goldberg whose telephone number is (703) 306-5817. The examiner can normally be reached Monday-Friday from 8:00 a.m. to 5:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (703) 308-1152. The fax number for this Group is (703) 305- 3014.

Any inquiry of a general nature should be directed to the Group receptionist whose telephone number is (703) 308-0196.

J. Goldberg

Jeanine Goldberg
May 14, 2003

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